membrane-bound. These activities can be solubilized by ultrasonic treatment under certain conditions, and work is under way to separate and purify these enzymes. Various factors that influence these reactions will be described. The formerly observed inhibition of ubiquinone biosynthesis by diphenylamine (Sugimura & Rudney, 1962) can now be located as an action on the first step, since it has been observed that the alkylation of POB is inhibited by this substance. Supplementation of other systems capable of synthesizing terpenoid quinones with this extract should be of great benefit in future work with cell-free systems and should help in clarifying some of the steps in the postulated pathways. This will be especially true where the rate-limiting step is the synthesis of polyprenyl pyrophosphates. We have obtained preliminary results with homogenates of rat kidney, liver and brain tissues which show the presence of a prenylated derivative of POB only after supplementation with the *M. lysodeikticus* extract.

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Polyprenols

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Polyprenols are those compounds with the general structure $H-[CH_2-C(CH_3)=CH-CH_2]_n-OH$ in which n is greater than 5. There is a variety of size (n may reach 24) and the shape varies according to the number and location of *cis*, *trans* and saturated isoprene residues.

The prenols are almost certainly formed as pyrophosphates by the usual condensation of isopentenyl pyrophosphate. The endogenous pool size of prenyl pyrophosphate or phosphate is always very small and if a prenol accumulates it is usually as the free alcohol.

The pyrophosphates of all-*trans*-prenols are probably formed primarily as the precursors of side chains of polyisoprenoid quinones. The natural all-*trans*-prenols that have been studied correspond in size to the all-*trans*-polyisoprenoid side chain of the endogenous quinone. Surprisingly, however, there is still no direct evidence that prenyl pyrophosphates do condense with aromatic precursors of the polyisoprenoid quinones.

Of the prenols isolated from tissues the all-*trans* forms are quantitatively relatively minor, the predominant prenols having mixed stereochemistry. Thus the leaves of most angiosperms yield small quantities (< 5mg./kg. of leaf) of solanesol (all-*trans*-prenol-9, $n = 9$), but considerably more (up to 2g./kg. of leaf) of *cis–trans*-polyprenols. Leaves of angiosperms contain a family of *cis–trans*-polyprenols, the individual members of which are made up of 9, 10, 11, 12 or 13 isoprene residues, usually with the prenol-11 or -12 predominating (Wellburn & Hemming, 1966*a*; Fukawa, Toyoda, Shimiza & Murohashi, 1966; Dunphy, Kerr, Pennock, Whittle & Feeney, 1967). Each polyprenol contains 3 internal *trans*-isoprene residues, the other internal residues and the OH-terminal isoprene residue (the α-residue) being *cis* (Wellburn, Stevenson, Hemming & Morton, 1967; Stone, Wellburn, Hemming & Pennock, 1967; Feeney & Hemming, 1967; Dunphy *et al.* 1967). It seems likely that these prenols are formed by *cis*-addition to geranylgeranyl pyrophosphate. On the other hand, the wood of the silver birch tree yields polyprenols containing from 6 to 9 isoprene residues (betulaprenols-6 to -9) which contain only 2 internal *trans*-residues and are thus probably formed by *cis*-addition to *trans–trans*-farnesyl pyrophosphate (Lindgren, 1965; Wellburn & Hemming, 1966*b*).

Although it is known that most of the polyprenols in leaves occur in chloroplasts, and that that remaining is associated with particulate fractions of the cell (Wellburn & Hemming, 1966*c*, 1967), nothing positive is known of their function. The

situation in bacteria is rather different. Elegant work has shown that the phosphate of a prenol-11 has an important role in the biosynthesis of bacterial wall components. The prenol phosphate appears to be located in the bacterial membrane and acts as an acceptor of sugar phosphates from water-soluble donor molecules inside the bacterium. The sugar molecule, while anchored to the prenol phosphate, may then have other sugar molecules added before being passed on to the insoluble polymer of the bacterial wall. This has been shown to be the case in the formation of peptidoglycan of *Micrococcus lysodeikticus* and *Staphylococcus aureus* (Higashi, Strominger & Sweeley, 1967) and in the biosynthesis of the *O*-antigen lipopolysaccharide of *Salmonella newington* (Wright, Dankert, Fennessey & Robbins, 1967). A similar process probably occurs in the biosynthesis of the wall polymannan of *M. lysodeikticus* (Scher, Lennarz & Sweeley, 1968), and there is tentative evidence for a similar role in teichoic acid biosynthesis in *Staphylococcus lactis* I3 (Baddiley, 1968). Thus in bacteria a role for prenol phosphates is well documented. Free prenols have not been found in bacteria other than the mevalonate-requiring lactobacilli. Thorne & Kodicek (1966) isolated from this source a prenol-11 that has one saturated isoprene residue. They called it bactoprenol. This prenol is the major metabolite of mevalonic acid, there being little or no poly-isoprenoid quinones or sterols. The stereochemistry of bacterial polyprenols (phosphates) is very similar to that of the leaf polyprenols.

Mammalian tissues contain a series of prenols much longer than those found in plants and bacteria. These are the dolichols containing 17, 18, 19, 20, 21 and 22 isoprene residues of which 2 internal residues are *trans* and the α-residue is saturated. They are probably formed by *cis*-addition to *trans–trans*-farnesyl pyrophosphate followed by saturation of the α-residue. The predominant polyprenol in rat tissue is dolichol-18 and in pig tissues is dolichol-19 (see e.g. Dunphy *et al.* 1967; Butterworth & Hemming, 1968).

Of the pig liver dolichols, about 60% are esterified to fatty acids, and of the unesterified dolichols in this tissue, most are associated with a mitochondrial fraction, obtained by differential centrifugation of a liver homogenate (Butterworth & Hemming, 1968).

Dolichols are also found in yeast. *Saccharomyces cerevisiae* contains predominantly dolichol-16 (Dunphy *et al.* 1967). The fungus *Aspergillus fumigatus* contains a family of hexahydropolyprenols. These are related to the dolichols in that the α-isoprene residue is saturated. In addition the ω- and ψ-residues (the two at the other end of the molecule) are also saturated. Of the remaining internal residues just two are in the *trans* configuration. The predominant prenol of *A. fumigatus* is hexahydroprenol-21 (Stone, Butterworth & Hemming, 1967). The proportion of hexahydroprenols esterified and the intracellular distribution of the free and esterified forms closely resembles the pattern for dolichols in pig liver (Stone & Hemming, 1968). Little is known of the function of dolichols and hexahydroprenols, but it is tempting to postulate a role for the phosphates in membranes similar to that for polyprenols in bacterial membranes.

Direct evidence is available from $^3H/^{14}C$ ratios of betulaprenols-6 to -9 and of rat liver dolichols biosynthesized from [2-^{14}C,(3*R*,4*R*)-4-$^3H_1$]- and [2-^{14}C,(3*R*,4*S*)-4-$^3H_1$]-mevalonic acid that the stereochemistry of each isoprene residue is fixed when it is formed by condensation of isopentenyl pyrophosphate. No isomerization appears to occur after this step (Gough & Hemming, 1967; D. Gough & F. W. Hemming, unpublished work). Similar studies on the biosynthesis of *Aspergillus* hexahydropolyprenols gave confirmatory results with 4*R*-mevalonic acid, but those prenols formed from 4*S*-mevalonic acid retained much less tritium than the number of *cis*- residues predicts (Stone & Hemming, 1967). This may well be due to a high activity of the reversible isopentenyl pyrophosphate isomerase relative to the activity of the prenol synthetase of this organism (a previously unforeseen problem in this sort of work). Biosynthetic studies of this type with leaf polyprenol and pig dolichols are difficult because of the low rate of incorporation of mevalonic acid into these molecules.

Only one cell-free system capable of synthesizing polyprenyl pyrophosphates has been described. This was by Allen, Alworth, McCrae & Bloch (1967), with *Micrococcus lysodeikticus*. The product corresponded in chain length to that of the endogenous menaquinone and was probably all-*trans*. It is interesting that *S. aureus* also contains a butanol-soluble enzyme capable of catalysing the phosphorylation of *cis–trans*-prenol-11 (Higashi, Siewert & Strominger, 1968). Polyprenol kinases have not been reported from any other source.

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Biological Function of Terpenoid Quinones

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The terpenoid quinones are widely distributed in Nature. In bacteria, plant and animal tissues the quinones are localized in the subcellular organelles involved in the biological processes of respiration and bioenergetics. Information concerning the role played by quinones in cellular metabolism has been obtained with bacterial systems after irradiation with light at 360nm. The sensitivity of the quinones to light at 360nm has made it possible to destroy the endogenous quinone without disruption of organelle structure or disassociation of the spatial arrangement of enzymes and coenzymes necessary for oxidative phosphorylation. Irradiation of extracts of *Mycobacterium phlei* was found to result in a loss of the endogenous quinone and in a loss of both oxidation and phosphorylation. The addition of the natural quinone, vitamin K_1, or closely related homologues was found to result in the restoration of oxidative phosphorylation. Although irradiation of the extracts was found to result in the loss of oxidative activity with succinate and NAD^+-linked substrates, restoration of activity by the addition of quinone occurred only with NAD^+-linked substrates. The succinate oxidase pathway appears to contain a light-sensitive water-soluble component that differs from the quinones.

The possibility arises that the restoration of activity by quinones is not directly related to a role in respiration but may be due to removal of a possible inhibitor formed during quinone destruction by light, or to a non-specific physical effect. This possibility, however, seems unlikely since restoration of oxidative phosphorylation occurs only with certain specific quinones. Certain substituted quinones, with physical and chemical properties similar to the quinones capable of restoring activity, were found to restore only oxidation and usually by an electron-transport by-pass reaction. In addition, the possible presence of an inhibitor formed during destruction of the endogenous quinone appears improbable since the first and third phosphorylative sites are operative in the irradiated system. Further, competitive inhibitors of vitamin K were shown to inhibit respiration in the untreated and quinone-restored system. Other methods of quinone depletion are available. Lipid extraction of the cell-free system results in a loss of oxidative phosphorylation. As in the irradiated system, restoration of activity occurs only on the addition of certain specific quinones.

The site of participation of the quinones in the respiratory chain has been determined with respiratory inhibitors and analysis of the reduction of the cytochromes in the irradiated system. For example, after irradiation, both NAD^+ and flavoproteins are reduced on the addition of substrate; however, the cytochromes remain in the oxidized state. Addition of a suitable quinone results in a reduction of the cytochromes. Thus, the quinone participates between flavin or non-haem iron and cytochrome *b* in the respiratory chain. Further, the quinone may function as a lipid cofactor since the rate of reduction and oxidation was found to be greater than the overall rate of oxidation from substrate to oxygen.

The ability of naphthaquinones to restore oxidation and phosphorylation to the light-treated system appears to depend on specific quinone structures. The naphthaquinones that restore both activities require the presence of a methyl group in the C-2 position and at least a C_5 unsaturated side chain in the C-3 position of the naphthaquinone nucleus. Studies of the structural requirements of the side chain for restoration of oxidative phosphorylation have indicated that the β–γ bond of the first isoprene unit must be unsaturated and that the second isoprenoid unit from the ring must be saturated as in MK_9(II-H). In addition, restoration of oxidative phosphorylation was only found to occur with the *trans* geometric isomers of MK_9(II-H) or vitamin K_1. In contrast, the *cis* isomers were found to partially restore oxidation but failed to restore phosphorylation.

Restoration of oxidation by quinones has been shown to be less specific than the requirements for restoration of oxidative phosphorylation. Although